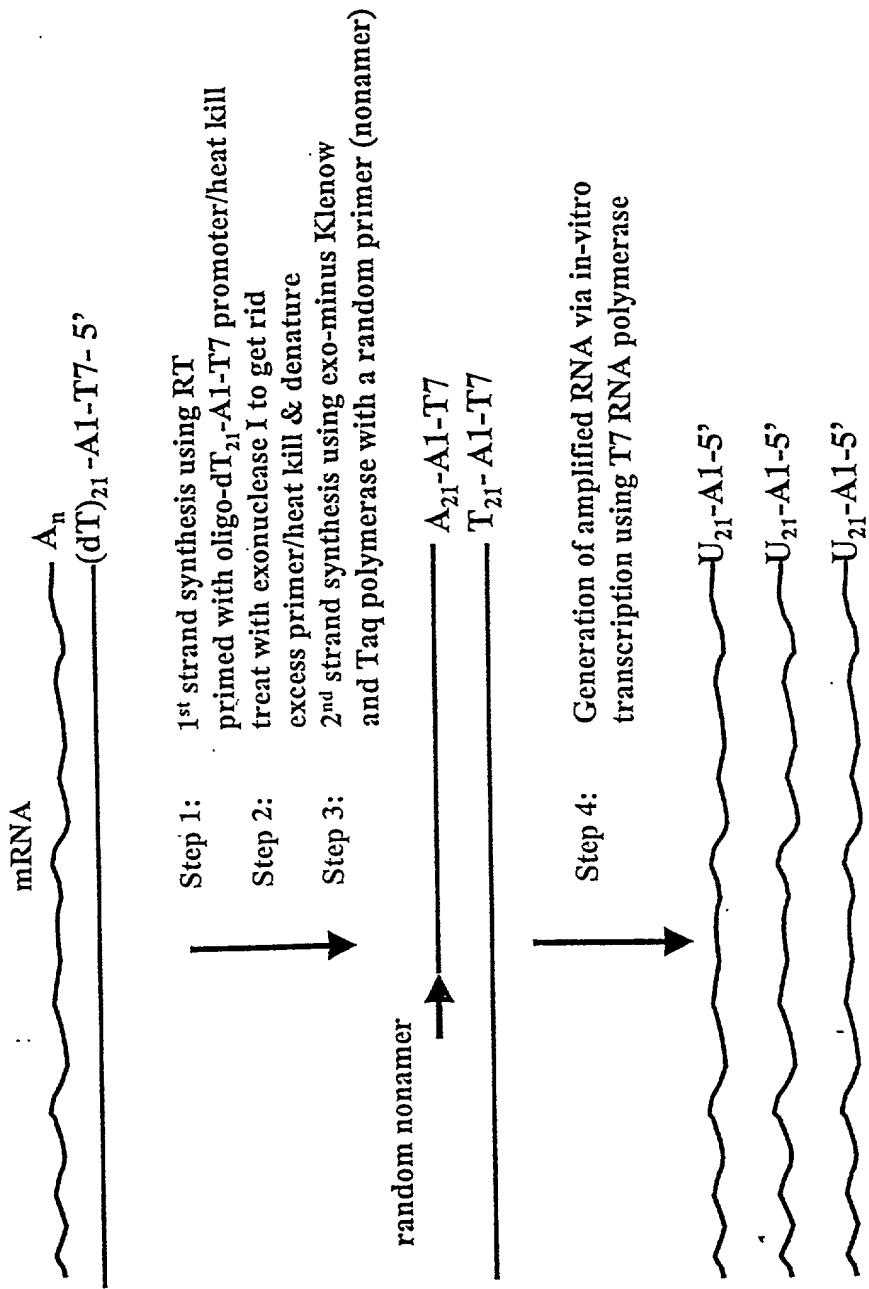


Figure 1 A

ROUND ONE:



ROUND TWO:

Figure 1B

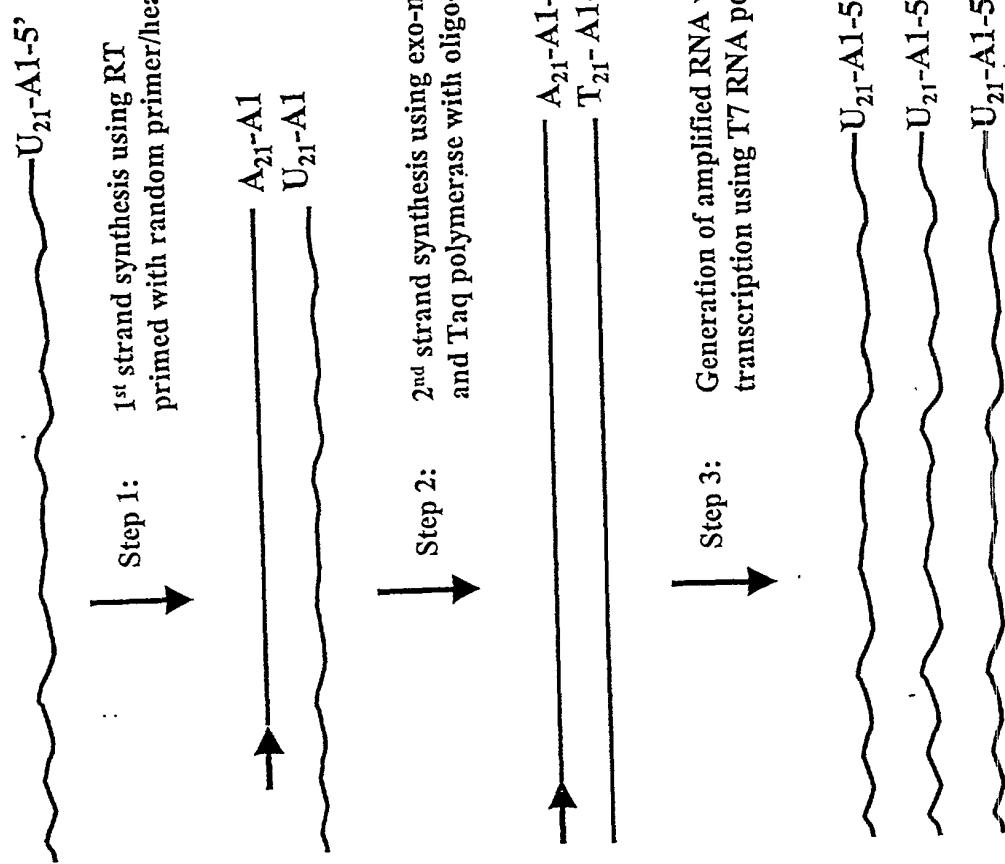


Figure 1C

ROUND TWO MODIFIED:

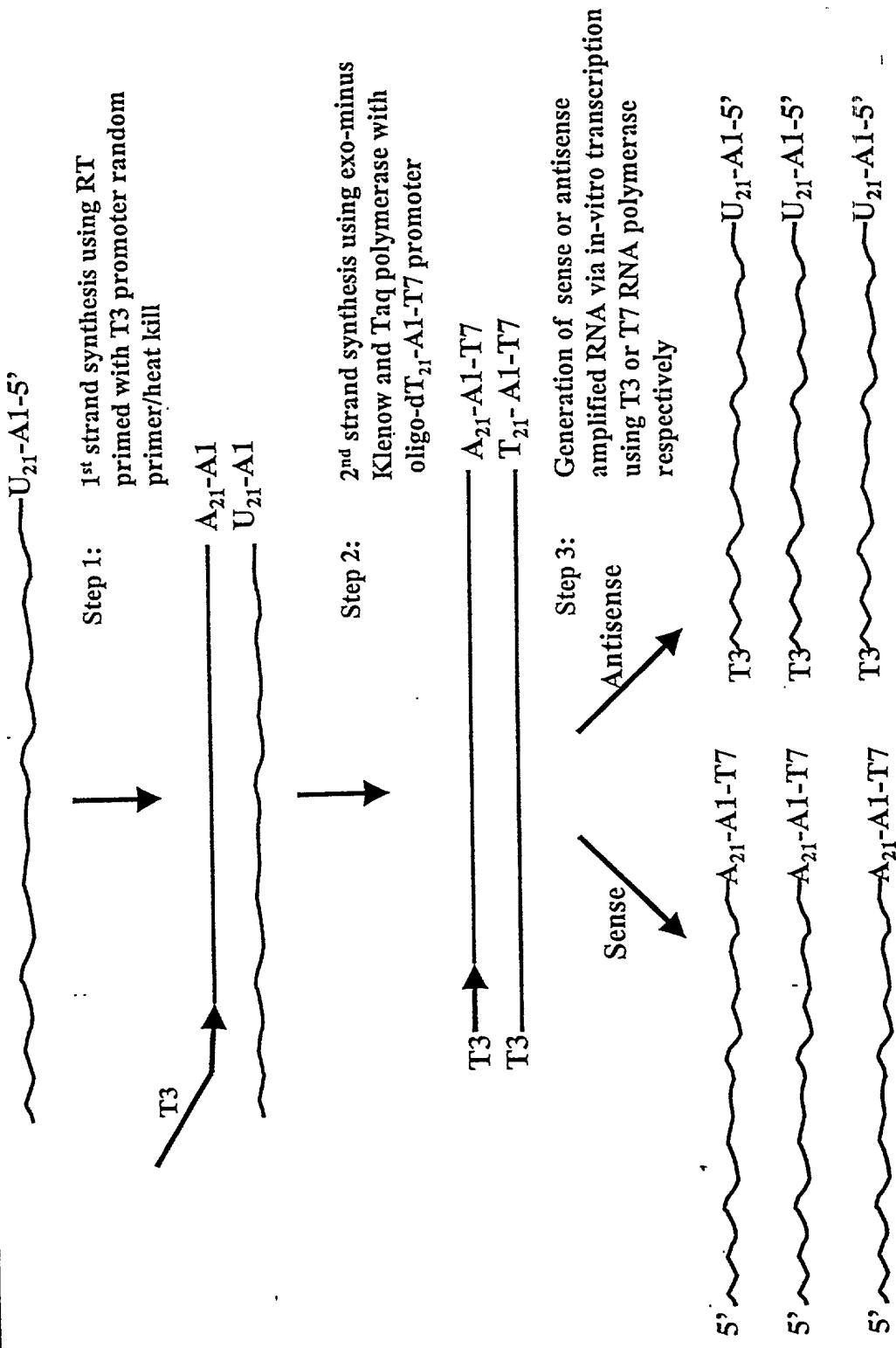


Figure 2A: Optimization of 2nd Strand Synthesis using Exogenous Primers

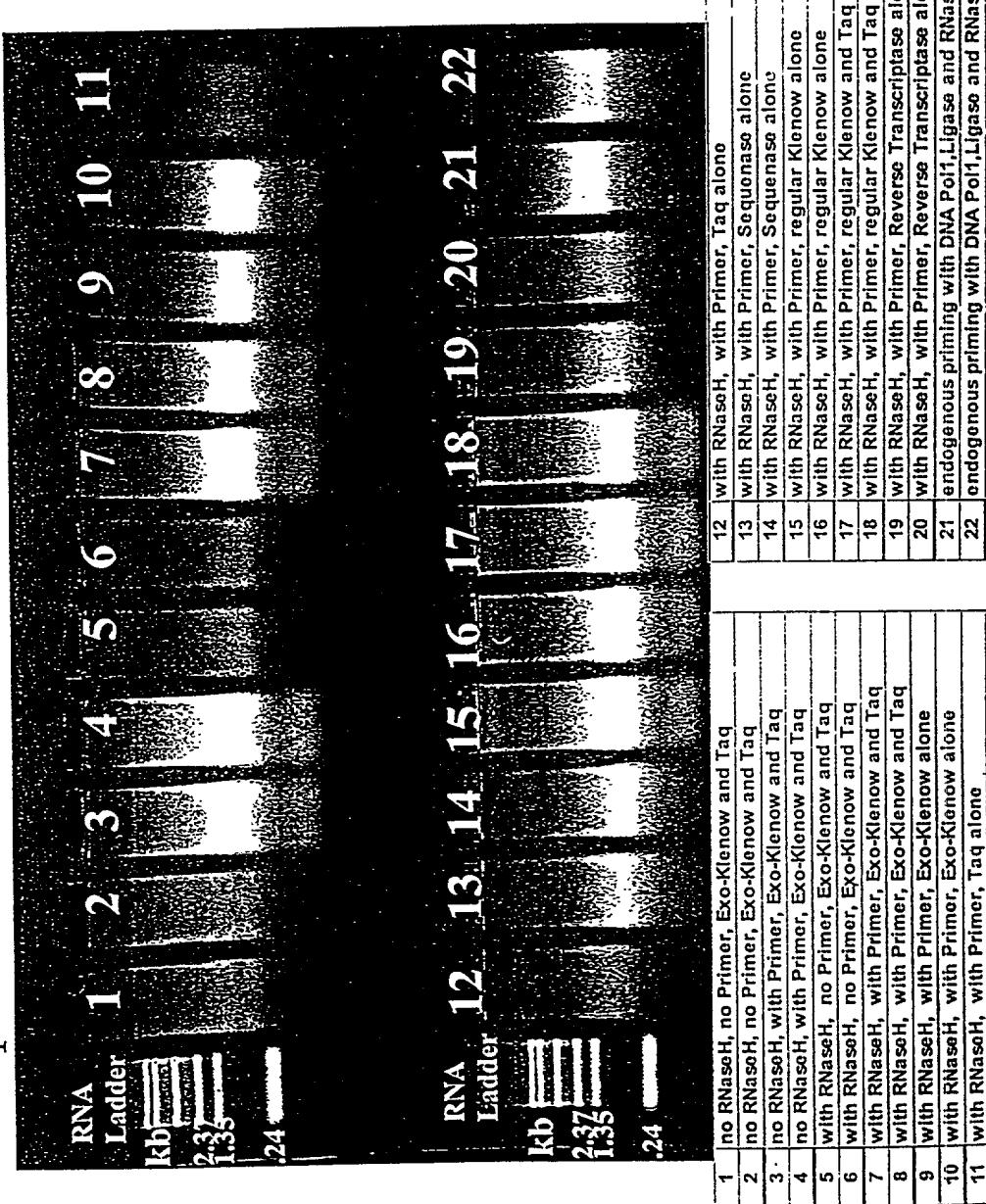


Figure 2B:Yields From Exogenous Priming of 2nd Strand Synthesis Using Different Enzymes

SAMPLES	Condition Tested	ug of amplified RNA
1	no RNaseH, no Primer, Exo-Klenow and Taq	3.6
2		3.4
3	no RNaseH, with Primer, Exo-Klenow and Taq	15.5
4		19.2
5	with RNaseH, no Primer, Exo-Klenow and Taq	3.4
6		3.0
7	with RNaseH, with Primer, Exo-Klenow and Taq	16.9
8		17.5
9	with RNaseH, with Primer, Exo-Klenow alone	18.7
10		16.8
11	with RNaseH, with Primer, Taq alone	2.8
12		3.6
13	with RNaseH, with Primer, Sequenase alone	9.0
14		10.4
15	with RNaseH, with Primer, regular Klenow alone	16.0
16		15.2
17	with RNaseH, with Primer, regular Klenow and Taq	13.7
18		15.2
19	with RNaseH, with Primer, Reverse Transcriptase alone	7.2
20		6.5
21	Eberwine1 endogenous priming method with DNA Pol1, Ligase and RNaseH	10.2
22	Eberwine2	11.7

Figure 2C: Comparison of Yields and Fold Amplification

SAMPLES	Condition Tested	ave (ug)	fold diff vs GH	est. fold amp*
1	no RNaseH, no Primer, Exo-Klenow and Taq	3.5	0.3	174
2				
3	no RNaseH, with Primer, Exo-Klenow and Taq	17.3	1.6	865
4				
5	with RNaseH, no Primer, Exo-Klenow and Taq	3.2	0.3	159
6				
7	with RNaseH, with Primer, Exo-Klenow and Taq	17.2	1.6	862
8				
9	with RNaseH, with Primer, Exo-Klenow alone	17.7	1.6	887
10				
11	with RNaseH, with Primer, Taq alone	3.2	0.3	161
12				
13	with RNaseH, with Primer, Sequenase alone	9.7	0.9	486
14				
15	with RNaseH, with Primer, regular Klenow alone	15.6	1.4	778
16				
17	with RNaseH, with Primer, regular Klenow and Taq	14.4	1.3	721
18				
19	with RNaseH, with Primer, Reverse Transcriptase alone	6.8	0.6	342
20				
21	Eberwine1 endogenous priming method with DNA Pol1, Ligase and RNaseH	11.0	1.0	548
22	Eberwine2			

*fold-amplification calculated as follows: (final μ g yield)/(0.020 μ g)
where 0.020 μ g is an estimate based on the assumption that 2% of 1 μ g
of total RNA (the amount of starting material) is poly(A) RNA

Figure 3A:

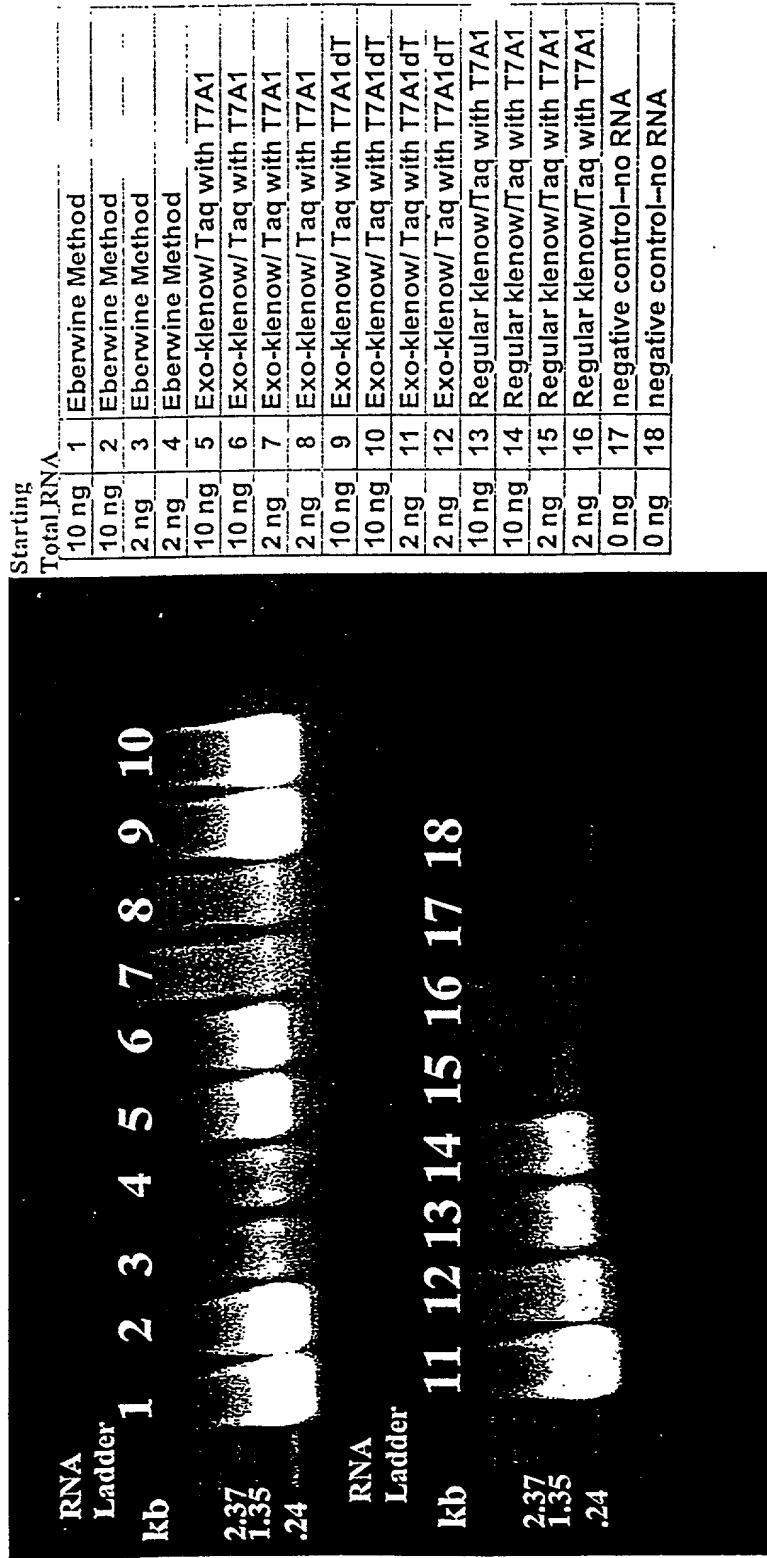


Figure 3B: